



Associations between food portion sizes, insulin resistance, VO2 max and metabolic syndrome in European adolescents: The HELENA study

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Abstract *Background and aims:* This study aims to examine the associations of food portion size (PS) with markers of insulin resistance (IR) and clustered of metabolic risk score in European adolescents.

Methods: A total of 495 adolescents (53.5% females) from the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study were included. The association between PS from food groups and homeostasis model assessment of insulin resistance (HOMA-IR) index, VO2 max, and metabolic risk score was assessed by multilinear regression analysis adjusting for several confounders. Analysis of covariance (ANCOVA) was used to determine the mean differences of food

Abbreviations: ANCOVA, Analysis of covariance; BMI, Body mass index; HELENA, Healthy Lifestyle in Europe by Nutrition in Adolescence; HOMA-IR index, Homeostasis model assessment of insulin resistance; Metabolic Syndrome, MS; PA, Physical activity; PS, Portion size; VO2 max, Maximal oxygen uptake.

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(MS);
Adolescents

PS from food groups by HOMA-IR cutoff categories by using maternal education as a covariable. *Results:* Larger PS from vegetables in both gender and milk, yoghurt, and milk beverages in males were associated with higher VO₂ max, while larger PS from margarines and vegetable oils were associated with lower VO₂ max ($p < 0.05$). Males who consumed larger PS from fish and fish products; meat substitutes, nuts, and pulses; cakes, pies, and biscuits; and sugar, honey, jams, and chocolate have a higher metabolic risk score ($p < 0.05$). Males with lower HOMA-IR cutoff values consumed larger PS from vegetables, milk, yoghurt, and milk beverages ($p < 0.05$). Females with lower HOMA-IR cutoff values consumed larger PS from breakfast cereals, while those with higher HOMA-IR cutoff values consumed larger PS from butter and animal fats ($p = 0.018$). *Conclusion:* The results show that larger PS from dairy products, cereals, and high energy dense foods are a significant determinant of IR and VO₂ max, and larger PS from food with higher content of sugar were associated with higher metabolic risk score.

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1. Introduction

During the past decades, increasing prevalence of type 2 diabetes mellitus and prediabetic stages such as insulin resistance (IR) or impaired fasting glucose has been reported in children and adolescents [1]. This increase seems to parallel the increase in the prevalence of obesity in these age groups [2]. Dietary factors are environmental determinants of both adiposity, IR, and the components of metabolic syndrome (MS) [3].

IRs improve after weight loss [4] and in the presence of high levels of physical activity (PA) [5]. Diet composition, in particular, carbohydrate type and amount and fat intake may also influence IR [6]. In children, it has been found that total energy, fat, saturated fat, and protein intakes were significant predictors of fasting insulin and quantitative insulin sensitivity check index (QUICKI), independent of body mass index (BMI), and age [7]. Several dietary factors could promote a positive energy balance [8] and thereby increase the risk for obesity and diabetes, including the following: excessive portion size (PS), with single large meals often approaching or exceeding individual daily energy requirements; palatability, emphasizing primordial taste preferences for sugar, salt, and fat; high energy density; and high glycemic load [9]. PS of many foods have been increasing in countries with a well-established industrialized food supply [10].

Increased consumption of margarine, sweets (candies, lollipops, jellies, and traditional fruit in heavy syrup), and savory snacks (chips, cheese puffs, and not home-made popcorn) was associated with high homeostasis model assessment of IR (HOMA-IR) index value in children and adolescents [11,12]. Additionally, it was shown previously that sugar intake in the form of sugar-sweetened beverages was associated with IR in adolescents [13]. Data from children indicated that short absorption time that follows the consumption of sugar may impair blood glucose control and may result in hyperinsulinemia and peripheral IR [14]. Frequent intake of obesogenic foods such as crackers,

chips, and cooked ham was observed in adolescents with MS [15]. Moreover, a ‘Western’ dietary pattern was associated with a greater risk for MS, among female adolescents [16].

The relationship between food PS and IR and the development of MS in children and adolescents have not been previously examined. Therefore, this study investigates the potential effect of food PS on IR and a quantitative score of metabolic risk in European adolescents.

2. Methods

2.1. Study design

A European multicentre cross-sectional study was performed (2006–2007) in adolescents aged 12.5–17.5 years from 10 cities to assess a Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) [17]. The main objective of the HELENA-Cross sectional was to obtain reliable and comparable data from randomly selected European adolescents ($n = 3528$, 52.3% females) by using wide relevant health and nutrition-related parameters that included the following: dietary intake, food choices and preferences, serum vitamin and mineral status, lipid and glucose metabolism, anthropometric measurements, PA and fitness, and genetic markers [18]. The inclusion criteria were participants who were free from any acute infection lasting less than 1 week before the inclusion process and were not concurrently involved in another clinical trial [19]. The exclusion criteria were participants not having information on age, gender, height, and weight; participants who were concurrently involved in another clinical trial, age more than 17.5 years or less than 12.5 years and having any acute infection lasting more than 1 week before the inclusion process [19]. More details about recruitment and sampling process are described elsewhere [19].

2.2. Study sample

Blood samples were obtained from around one-third of patients, following the same randomization criteria as those for the whole sample. Out of 3528 adolescents included in the HELENA study, blood samples were obtained from one-third (1089) of the adolescents as was foreseen in the protocol. A total of 1188 adolescents (33.7%) did not have information for the 24-hr. Also, 1198 adolescents who were considered over-reporters (173) and under-reporters (526), according to the approach of Goldberg et al. [20], were excluded. Out of those with valid dietary data, 647 participants were excluded, as they had no data on glucose, insulin, and HOMA-IR index, skinfold thickness, systolic blood pressure, triglycerides, maximal oxygen uptake (VO₂ max), maternal education, or PA. Finally, 495 (265 females) adolescents were included in the present analysis.

The study was performed following the ethical guidelines of the Declaration of Helsinki 1964 (revision of Edinburgh, 2000), the Good Clinical Practice, and the legislation about clinical research in humans in each of the participating countries. The protocol was approved by the Human Research Review Committees of the involved centres [21]. Moreover, a written informed consent was obtained from participating adolescents and their parents [22].

2.3. Questionnaires

The education level of the adolescent's mothers was adapted from the International Standard Classification of Education (ISCED) [23] and reported as primary education, lower secondary education, higher secondary education, and higher education/university degree. In this study, the two lowest levels have been merged into one group called lower level of education, in addition to higher level of education. More details have been reported elsewhere [24].

2.4. Physical examination

Measurements were taken 3 times by trained researchers in each city. A training session was conducted by the coordinator of HELENA, with the 10 field workers who planned to perform anthropometric measurements. The aim of the training was to familiarize researchers with the exact protocol to be used and to perform the 1st approach to assess the intra-observer technical error. Then, a workshop was organized that aims to assess the intra-observer (2nd time) and inter-observer (1st time) technical error of measurements (TEMs < 1) and the reliability (> 90%) of anthropometry and BIA measurements. All the anthropometric variables were measured in order, and the same measurements were then repeated two more times [25].

Weight was measured with an electronic scale (model 871; SECA, Hamburg, Germany) to the nearest 0.05 kg, and

height was measured with a telescopic height measuring instrument (model 225; SECA, Hamburg, Germany) to the nearest 0.1 cm. All measurements were performed in underwear and barefoot [25]. BMI was calculated as body weight (kg) divided by the height (m) squared (kg/m²). The obesity status was classified using the International Obesity Task Force scale [26]. Skinfold thickness was measured to the nearest 0.2 mm in triplicate in the right side at biceps, triceps, subscapular, suprailiac, thigh, and medial calf with a Holtain Caliper (Crymmych, Wales, UK). The sum of six skinfold thickness was used as an indicator of total body fat [27].

2.5. Physical activity measurement

Accelerometers (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton Beach, FL, USA) were used to obtain an objective measurement of PA. The devices were placed on the lower back of the participants under the clothes using an elastic belt for seven sequent days. Instructions were given to participants when they wake up to wear the instrument and remove it for water-based activities and sleeping [28]. Data were downloaded to the computer using manufacturer software and analyzed later by software based on Visual Basic. Time spent in moderate and vigorous physical activity (MVPA) was determined using the cutoff point of 2000 cpm to generate the various indices; the number of days per week was multiplied by minutes per day, to calculate minutes per week for each activity [29]. More detailed information has been reported elsewhere [28].

2.6. Blood samples

Briefly, fasting blood samples were collected by venepuncture at school between 8:00 and 10:00 after a 10-h overnight fast. Whole blood samples for the hemogram were sent directly to the local laboratory of each country to be analyzed. Concentrations of triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and glucose were measured in fresh serum enzymatically on the Siemens Dimension RxL Max Integrated Chemistry System (Dade Behring, Schwalbach, Germany) using the manufacturer's reagents and instructions at the University Hospital in Bonn (Germany). TC/HDL-c ratio was calculated. Insulin was measured by a solid-phase two-site chemiluminescent immunometric assay with an Immulite 2000 analyzer (DPC Biermann GmbH, Bad Nauheim, Germany). More details about blood handling procedures have been described elsewhere [30]. The intra-assay coefficients of variation were < 3.3%, and the inter-assay coefficients were < 3.9% for all parameters.

2.7. Cardiorespiratory fitness

Cardiorespiratory fitness was measured by the progressive 20-m shuttle run test [31]. This test required participants to run back and forth between two lines set 20 m apart

following a running pace determined by audio signals and with an initial speed of 8.5 km h⁻¹ increasing by 0.5 km h⁻¹ every minute (1 min equals 1 stage). The test is finished when the adolescent failed to reach the end lines concurrent with the audio signals on two consecutive occasions, and the final score was computed as the number of stages completed (precision of 0.5 stages). Maximal oxygen uptake (VO₂ max) was estimated using the formula described by Léger et al. (1984).

2.8. Metabolic risk score

The HOMA-IR index was calculated as fasting insulin [(pmol/l)/6.945] * fasting glucose [(mmol/l)/22.5] [32]. The cutoff value for HOMA-IR was based on the 90th percentile. A QUICKI was calculated as QUICKI = 1/log insulin (IU/mL) + log glucose (mg/dl) [32]. Systolic blood pressure was measured with an automatic oscillometric device (M6, HEM-7001-E, Omron). A continuous score of clustering metabolic risk factors was computed using the following variables: systolic blood pressure, triglycerides, TC/HDL-c ratio, HOMA-IR index, the sum of six skinfolds, and VO₂ max. Z-scores were calculated for each risk factor variable by age and gender, and then all individual Z-scores were summed to create a clustered risk score based on the one by Andersen et al. [33], that has been used in the previous HELENA study [12].

2.9. Dietary assessment

The HELENA Dietary Assessment Tool (HELENA-DIAT) was used to assess adolescents dietary consumption; this software was used as self-administered, computerized 24-h recall, developed and validated originally in Flemish adolescents, and then in the HELENA-CSS [34]. HELENA-DIAT is based on previous day assessments of the intake from six meal occasions (breakfast, morning snack, lunch, afternoon snack, evening meal, and evening snack). The two nonconsecutive 24-h recalls performed on one convenient weekday and one weekend day. A well-trained dietitian was present to assess the adolescent in case they need any help to complete the diet 24-hr recall.

A total of 800 photographs were available in the HELENA-DIAT program. The participants were able to select one of the amounts that appeared in a photograph or indicate that they consume less or more than the amount appeared on the computer. In addition, they were able to type the consumed amount for each food item in a text box. Moreover, the participants were able to remove or modify the selected items at any time. Moreover, foods that can be measured with household tools like cups, several portions appeared on the screen, so that the participants can select the consumption amount by clicking directly on the portion. In case some foods usually eaten in combination with other items such as french fries and mayonnaise, a box was shown on the screen to remind them to include this item [34].

2.10. Selection of food groups

Based on the European food groups classification system, about 4179 foods and beverages, in the form of recipes or as individual food, were aggregated into food groups [34]. In our study, we excluded the foods that were very infrequently consumed from the analysis: products for special nutrition use, soya beverages, and miscellaneous due to their very low consumption (reported by less than 15% of the participants). Furthermore, the daily diet was divided into 11 food groups based on their nutritional composition: (1) water, (2) bread and cereal, (3) grains and potatoes, (4) fruit, (5) vegetables, (6) milk, milk desserts, and yogurt, (7) cheese, (8) meat/fish/eggs/vegetarian substitutes, (9) spread and cooking fat (10) low-nutrient, energy-dense foods (e.g., chocolate, sugar products, biscuits, pies, savoury snacks, creams, and confectionery), and (11) low-nutrient, energy-dense drinks (e.g., carbonated soft drinks, juices, and alcoholic drinks). Milk products and cheese were allocated to different food groups because of the important difference in fat content.

2.11. Portion size calculation

PS was calculated by dividing the intake in grams (g) of the items included in the group and reported to be consumed during the 24 h-recall, by the number of eating occasions of these consumed items. In this study, the average amount of PS was calculated from the two days included in the 24 h-recall by each eating occasion. For instance, if an individual consumed 100 g of meat for lunch in the first day and 100 g in the lunch for the second day, then his/her PS at lunch from this food item was 100 g, and if the individual consumed 100 g of meat only in lunch and did not consume meat in any other meal, his/her PS was 100 g. Several studies of food PS effect on overweight in children and adults have used the same methodology [35,36]. Thus, these data represent per consumer averages, not per capita averages, and it is used to show the average change on the PS for those who consume a certain item. Therefore, to analyze a specific food group only participants who consumed this food group were included in the analysis.

2.12. Statistical analysis

Descriptive analysis of mean and standard deviation for general characteristics were presented with Student's t-test for continuous variables. Chi-square test was used to assess the difference of categorical variables between genders. To achieve normality Johnson transformation has been performed for VO₂ max, HOMA-IR index, and metabolic risk score. Sensitivity analysis was carried out in order to consider age or pubertal stage in adjustment. Interaction products of gender and markers of body fat in the association between PS from food groups with markers of IR were calculated. Since an interaction effect was observed for gender, all the analyses were performed separately for females and males. Analysis of covariance

(ANCOVA) was also used to determine the mean differences and standard deviations of food PS from the studied food groups by HOMA-IR cutoff categories and metabolic risk score median cutoff categories between gender by using maternal education as covariable for all participants.

The association between PS from food groups as independent variables and HOMA-IR, systolic blood pressure, triglycerides, TC/HDL-c ratio, the sum of six skinfolds and VO2 max, and the metabolic risk score as dependent variables was assessed by multilinear regression analysis. All regression models were adjusted for age, maternal education, PA, total energy intake, BMI, and city as dummy variable. To avoid multiple testing, all analysis were performed individually. The analyses were conducted using IBM-SPSS (v25, SPSS Inc., Chicago, IL, USA), and the level of significance was set to 0.05.

3. Results

3.1. General characteristics of study participants

Sample descriptive characteristics by gender are presented in [Table 1](#). A total of 495 adolescents aged between

Table 1 Descriptive characteristics of the study sample.

General characteristics	All participants (n = 495)		p-value
	Males (n = 230)	Females (n = 265)	
	Mean (SD)	Mean (SD)	
Age	14.7 (1.3)	14.7 (1.2)	0.314
Maternal education (n, %) ^a			0.914
Low	69 (30.0%)	90 (33.9%)	
High	161 (70.0%)	175 (66.1%)	
BMI (kg/m ²)	20.6 (3.4)	20.8 (3.3)	0.789
BMI categories (n,%)			0.090
Normal weight	189 (82.2%)	229 (86.4%)	
Overweight	30 (13.0%)	26 (9.8%)	
Obesity	11 (4.8%)	10 (3.8%)	
Glucose (mg/dL)	91.9 (7.2)	88.3 (6.2)	0.047
Triglycerides (mg/dL)	65.4 (31.9)	70.8 (30.7)	0.730
Total cholesterol (mg/dL)	152.4 (25.4)	166.2 (27.1)	0.198
Insulin (μIU/mL)	8.9 (6.9)	10.0 (6.8)	0.899
HOMA-IR index ^b	2.1 (1.7)	2.2 (1.7)	0.811
QUICKI	0.4 (0.03)	0.3 (0.03)	0.010
SBP (mm Hg)	119.2 (13.0)	112.2 (11.5)	0.102
VO2max (ml/kg/min)	53.2 (7.9)	36.5 (6.4)	0.002
Sum of skinfold thickness (mm)	22.4 (11.3)	30.2 (11.8)	0.216
Waist circumference (cm) ^b	72.9 (8.3)	69.9 (7.2)	0.164
Metabolic risk score ^c	-0.81 (3.4)	0.75 (2.7)	0.060
TC/HDL ratio	2.9 (0.6)	2.9 (0.6)	0.680

All values are mean ± standard deviation, or ^a percentage. BMI: body mass index; HOMA-IR index: homeostasis model assessment for insulin resistance; SBP: systolic blood pressure; TC/HDL-c: total cholesterol/high-density lipoprotein cholesterol; VO2max: maximal oxygen uptake. Non transformed data are presented in this table, but analyzes were performed with ^b and ^c Johnson transformation. Level of significance was set to 0.05.

12.5 and 17.5 years old were included in this study. More than half (53.5%) of the participants were females. Males had significantly higher mean glucose ($p = 0.047$), QUICKI ($p = 0.010$), and VO2 max ($p = 0.002$), than females.

3.2. Association between PS from various food groups and HOMA-IR index and metabolic risk score components (systolic blood pressure, triglycerides, TC/HDL-c ratio, sum of six skinfolds, and VO2 max) by gender

The results are showing no significant association between food PS and HOMA-IR index ([Table 2](#)), and all of metabolic risk score components except VO2 max in both genders ([Supplementary Tables S1–S4](#)) after adjustment for age, maternal education, PA, total energy intake, BMI, and city.

3.3. Association between PS from various food groups and VO2 max stratified by gender

The result from [Table 3](#) shown that in males, larger PS from vegetables ($\beta = 0.001$; $p = 0.048$), milk, yoghurt, and milk beverages ($\beta = 0.002$; $p = 0.026$) were associated with higher VO2 max; while larger PS from margarines and vegetable oils ($\beta = -0.004$; $p = 0.025$) were associated with lower VO2 max. In females, larger PS from vegetables ($\beta = 0.001$; $p = 0.007$) were associated with higher VO2 max, taking in consideration the adjustment for age, maternal education, PA, total energy intake, BMI, and city.

3.4. Association between PS from various food groups and metabolic risk score

[Table 4](#) illustrates the results of multilinear regression model by gender using metabolic risk score categories as a dependent variable and PS of food as independent variables. The model was adjusted for age, maternal education, PA, total energy intake, BMI, and city. The results indicates that males with larger PS consumption from fish and fish products ($\beta = 0.007$; $p = 0.015$); meat substitutes, nuts, and pulses ($\beta = 0.018$; $p = 0.032$); cakes, pies, and biscuits ($\beta = 0.005$; $p = 0.010$); sugar, honey, jams, and chocolate ($\beta = 0.005$; $p = 0.009$) have higher metabolic risk score. Females with only larger PS from cakes, pies, and biscuits ($\beta = 0.005$; $p = 0.030$) have higher metabolic risk score.

3.5. Relationship between PS mean intake from food groups and HOMA-IR cutoff categories by gender

PS mean intake characteristics were obtained from the different food groups and HOMA-IR cutoff categories using mother's education as covariable (ANCOVA) for all participants ([Table 5](#)). The results indicate that males with lower HOMA-IR cutoff consumed higher mean PS from vegetables ($p = 0.036$) and milk, yoghurt, and milk beverages ($p = 0.040$). In the same line, females with lower HOMA-

Table 2 The association between food PS and HOMA-IR index in a selected sample of European adolescents, by gender.

Food Groups (g/day)	HOMA-IR index							
	Males (n = 230)				Females (n = 265)			
	β	95% CI		P-value	β	95% CI		P-value
	Lower	Upper		Lower	Upper			
Bread and Cereals								
<i>Bread and rolls</i>	0.011	-0.002	0.003	0.589	-0.003	-0.007	0.001	0.102
<i>Breakfast cereals</i>	-0.010	-0.022	0.003	0.212	-0.007	-0.014	0.001	0.051
Grains and potato								
<i>Rice and other grains</i>	0.050	-0.004	0.009	0.500	-0.012	-0.002	0.005	0.847
<i>Starch roots, potatoes</i>	-0.001	-0.003	0.002	0.512	-0.001	-0.002	0.001	0.561
<i>Pasta</i>	0.004	-0.001	0.003	0.589	0.002	0.000	0.004	0.087
Fruits	0.051	-0.010	0.006	0.052	0.007	-0.005	0.003	0.507
Vegetables	0.002	0.001	0.003	0.066	0.002	-0.001	0.002	0.654
Milk, milk desserts and yogurt								
<i>Milk, yoghurt, and milk beverages</i>	0.001	-0.005	0.001	0.065	0.002	-0.001	0.002	0.160
<i>Desserts and puddings milk based</i>	-0.007	-0.012	0.003	0.067	-0.004	-0.004	0.003	0.164
Cheese	0.001	-0.002	0.005	0.792	-0.025	-0.008	0.002	0.614
Meat/poultry/fish/eggs								
<i>Meat and poultry</i>	0.002	-0.003	0.002	0.546	0.003	-0.002	0.005	0.253
<i>Fish and fish products</i>	0.013	-0.004	0.001	0.298	0.005	-0.001	0.009	0.845
Eggs	-0.015	-0.008	0.007	0.771	0.002	-0.003	0.002	0.524
<i>Meat substitutes, nuts, pulses</i>	-0.045	-0.055	0.096	0.105	-0.010	-0.009	0.018	0.096
Spread and cooking fats								
<i>Margarines and vegetable oils</i>	0.011	-0.008	0.008	0.912	0.006	-0.009	0.016	0.395
<i>Butter and animal fats</i>	0.023	-0.005	0.007	0.136	0.019	-0.019	0.005	0.950
Low nutrient energy- dense food								
<i>Cakes, pies, biscuits</i>	0.012	-0.010	0.009	0.104	0.001	-0.002	0.002	0.600
<i>Savoury snacks</i>	-0.003	-0.004	0.004	0.507	0.006	-0.002	0.012	0.213
<i>Sugar, honey, jams, chocolate</i>	-0.005	-0.002	0.006	0.460	-0.004	-0.001	0.006	0.105
<i>Sauces and creams</i>	0.003	-0.008	0.005	0.136	0.019	-0.020	0.000	0.248
Low nutrient energy- dense drinks								
<i>Carbonated soft/isotonic drinks</i>	0.001	-0.003	0.003	0.391	0.006	0.002	0.009	0.125
<i>Fruit and vegetables juices</i>	0.008	-0.004	0.009	0.235	0.003	-0.002	0.011	0.161

β : regression coefficient. CI: confidence interval; Adjusting for confounders: age, maternal education, PA, total energy intake, BMI and city. Level of significance was set to 0.05.

IR cutoff consumed higher mean PS from breakfast cereals ($p = 0.010$). Contrary, females with higher HOMA-IR cutoff, consumed higher mean PS from butter and animal fats ($p = 0.018$).

3.6. Relationship between PS mean intake from food groups and metabolic risk score median cutoff categories by gender

The results indicate that no significant relationship between food PS and metabolic risk score median cutoff categories ([Supplementary Table S5](#)).

4. Discussion

The main results suggest that there is an association between PS of some food groups and a metabolic risk score in adolescence. Specifically, we identified that larger PS from cakes, pies, biscuits in males and females were associated with higher metabolic risk score. Meanwhile, PS from fish; meat substitutes, nuts, and pulses; and sugar, honey, jams, and chocolate were associated with a higher metabolic risk score in males, considering potential confounders, such as PA, total energy intake, BMI, city, and maternal education.

4.1. Portion size of specific food groups and metabolic risk score components

Out of the components of the metabolic risk score, significant results were found only for VO₂ max, a marker of cardiorespiratory fitness. We found that larger PS from vegetables in both genders and milk, yoghurt, and milk beverages were associated with higher VO₂ max in males, while larger PS from margarines and vegetable oils were associated with lower VO₂ max in males. In general, fruit and vegetables are one of the most abundant source of natural flavonoids Quercetin [37]. Noteworthy, these compounds play an important role as antioxidant and anti-inflammatory activity, in addition to the most prominent role which is the ability to increase mitochondrial biogenesis in both muscle and brain in mice [38]. In adults, low doses of the naturally occurring dietary flavonoid quercetin were associated with a modestly higher VO₂ max [39]. Similarly, in young adult, it has been observed a significantly higher levels of VO₂ max in the vegetarian compared with omnivores [40]. Moreover, it has been found that adolescents with the highest cardiorespiratory fitness are the most active and tend to consume higher fruits and vegetables [41].

Table 3 The association between food PS and VO2 max in a selected sample of European adolescents, by gender.

Food Groups (g/day)	VO2 Max							
	Males (n = 230)				Females (n = 265)			
	β	95% CI		P-value	β	95% CI		P-value
	Lower	Upper		Lower	Upper			
Bread and Cereals								
<i>Bread and rolls</i>	0.006	-0.005	0.004	0.605	-0.002	-0.022	-0.001	0.990
<i>Breakfast cereals</i>	0.002	-0.002	0.002	0.150	0.003	-0.003	0.010	0.110
Grains and potato								
<i>Rice and other grains</i>	0.006	-0.001	0.003	0.104	0.000	-0.002	0.001	0.376
<i>Starch roots, potatoes</i>	0.022	-0.008	0.004	0.467	0.005	-0.005	0.015	0.293
<i>Pasta</i>	0.031	-0.023	0.014	0.450	0.021	-0.013	0.011	0.219
Fruits	0.001	-0.002	0.005	0.232	0.002	-0.001	0.004	0.154
Vegetables	0.033	-0.002	0.015	0.038	0.026	-0.007	0.009	0.012
Milk, milk desserts and yogurt								
<i>Milk, yoghurt, and milk beverages</i>	0.009	-0.001	0.008	0.006	0.000	0.000	0.001	0.559
<i>Desserts and puddings milk based</i>	0.004	-0.010	0.000	0.079	0.005	-0.004	0.003	0.374
Cheese	0.001	-0.009	0.002	0.090	0.002	-0.005	0.003	0.754
Meat/poultry/fish/eggs								
<i>Meat and poultry</i>	0.009	-0.006	0.002	0.150	0.030	-0.015	0.009	0.059
<i>Fish and fish products</i>	0.005	-0.013	0.003	0.420	0.003	-0.005	0.009	0.400
Eggs	0.001	-0.006	0.008	0.772	0.001	-0.001	0.003	0.445
<i>Meat substitutes, nuts, pulses</i>	0.002	-0.003	0.007	0.205	-0.001	-0.003	0.004	0.306
Spread and cooking fats								
<i>Margarines and vegetable oils</i>	-0.008	-0.012	0.009	0.015	0.003	-0.003	0.010	0.267
<i>Butter and animal fats</i>	0.004	-0.001	0.012	0.508	0.002	-0.032	0.005	0.508
Low nutrient energy- dense food								
<i>Cakes, pies, biscuits</i>	0.000	-0.001	0.002	0.692	-0.002	-0.004	0.000	0.067
<i>Savoury snacks</i>	0.004	-0.003	0.010	0.288	-0.007	-0.014	0.001	0.077
<i>Sugar, honey, jams, chocolate</i>	-0.001	-0.003	0.002	0.512	-0.001	-0.002	0.001	0.561
<i>Sauces and creams</i>	0.000	-0.003	0.002	0.687	-0.009	-0.003	0.002	0.937
Low nutrient energy- dense drinks								
<i>Carbonated soft/isotonic drinks</i>	0.000	-0.003	0.002	0.686	0.002	0.000	0.004	0.087
<i>Fruit and vegetables juices</i>	0.000	-0.001	0.001	0.701	0.000	-0.001	0.001	0.617

β : regression coefficient. CI: confidence interval; Adjusting for confounders: age, maternal education, PA, total energy intake, BMI and city. Level of significance was set to 0.05

4.2. Portion size of specific food groups and metabolic risk score

Regarding metabolic risk score, we found that larger PS from cakes, pies, biscuits in males and females were associated with higher metabolic risk score. PS from fish and fish products; meat substitutes; nuts, pulses, and sugar; and honey, jams, and chocolate were associated with higher metabolic risk score in males. In a previous study in adolescents, significant differences were found, in the consumption of pretzels, chips, ham, and burgers between the group with MS and those who did not have the syndrome [15].

In adult men, it has been noticed that seafood consumption was significantly associated with elevated high-sensitivity C-reactive protein levels, after adjustment for age, PA, and BMI [42]. However, contrary to our results, a study found that low-fat meats and fish and fish products consumption was negatively associated with the inflammatory response in adults [43]. In this study, higher intake of fish and fish products was associated with higher metabolic risk score. The benefits of increased fish and fish products consumption on health have been associated to its content of omega 3 and PUFA, but fish consumption contributes with considerable amounts of other nutrients

that may have an effect on the metabolic score [44]. The possible explanation of our results is that processed fish such as fish cakes, fish balls, fish pudding, and fish fingers are made from lean fish filet mixed with other ingredients like: milk and flour; moreover, it has been found that these products contain free or added sugars and saturated and trans-fats that makes them high energy dense food, these fish products represent nearly 40% of the total fish consumption [45]. The lack of health benefits from processed fish may partly be explained by a reduction of some of the nutrients present during the processing such as deep-fried, fried, boiled, or minced, and therefore, they contain a higher amount of total fat. Additionally, these products previously contained trans-fatty acids that are known to be associated with lowered HDL-level [46].

Moreover, an increased frequency of consumption of fruits and vegetables, and dairy products decreased the probability of having MS in adolescents [47], while the probability of having MS increased along with the consumption of solid hydrogenated fat, and bread made with white flour in both genders [47]. Similarly, a systematic review focusing on European dietary patterns and MS from various age groups concluded that higher intake of meat or meat products, desserts, and sugar-sweetened beverages, which are considered as a good source of

Table 4 The association between food PS and a metabolic risk score in a selected sample of European adolescents, by gender.

Food Groups (g/day)	Metabolic risk score							
	Males (n = 230)				Females (n = 265)			
	B	95% CI		P-value	β	95% CI		P-value
	Lower	Upper			Lower	Upper		
Bread and Cereals								
<i>Bread and rolls</i>	0.000	-0.002	0.001	0.435	0.003	-0.002	0.001	0.695
<i>Breakfast cereals</i>	-0.003	-0.002	0.007	0.209	0.005	-0.003	0.012	0.210
Grains and potato								
<i>Rice and other grains</i>	-0.001	-0.002	0.001	0.380	0.001	-0.001	0.003	0.461
<i>Starch roots, potatoes</i>	-0.001	-0.001	0.003	0.215	0.000	-0.002	0.001	0.522
<i>Pasta</i>	-0.006	-0.002	0.001	0.758	0.000	-0.002	0.001	0.666
Fruits	0.003	-0.001	0.001	0.936	0.000	-0.001	0.000	0.428
Vegetables	0.011	-0.012	0.002	0.058	0.001	-0.008	0.002	0.097
Milk, milk desserts and yogurt								
<i>Milk, yoghurt, and milk beverages</i>	-0.002	0.000	0.001	0.056	0.002	-0.010	0.001	0.559
<i>Desserts and puddings milk based</i>	-0.001	-0.002	0.003	0.456	-0.002	-0.005	0.000	0.077
Cheese	0.002	-0.001	0.004	0.298	0.001	-0.003	0.004	0.285
Meat/poultry/fish/eggs								
<i>Meat and poultry</i>	0.000	-0.001	0.000	0.157	-0.002	-0.001	0.001	0.959
<i>Fish and fish products</i>	0.005	-0.012	0.020	0.007	0.001	-0.003	0.002	0.593
Eggs	0.001	-0.006	0.008	0.772	0.001	-0.001	0.003	0.445
<i>Meat substitutes, nuts, pulses</i>	0.031	0.006	0.050	0.001	0.004	-0.004	0.014	0.307
Spread and cooking fats								
<i>Margarines and vegetable oils</i>	0.013	-0.005	0.002	0.050	0.001	-0.003	0.010	0.267
<i>Butter and animal fats</i>	0.000	-0.009	0.008	0.848	-0.002	-0.012	0.008	0.698
Low nutrient energy-dense food								
<i>Cakes, pies, biscuits</i>	0.004	0.001	0.023	0.036	0.004	-0.012	0.003	0.020
<i>Savoury snacks</i>	0.015	-0.004	0.019	0.147	0.025	-0.002	0.012	0.184
<i>Sugar, honey, jams, chocolate</i>	0.023	0.009	0.028	0.009	-0.001	-0.007	0.006	0.728
<i>Sauces and creams</i>	-0.003	-0.002	0.002	0.974	-0.006	-0.002	0.002	0.945
Low nutrient energy- dense drinks								
<i>Carbonated soft/isotonic drinks</i>	0.005	-0.001	0.009	0.763	0.003	-0.004	0.001	0.254
<i>Fruit and vegetables juices</i>	-0.000	-0.001	0.012	0.101	0.002	-0.002	0.000	0.243

β : regression coefficient. CI: confidence interval; Adjusting for confounders: age, maternal education, PA, total energy intake, BMI and city. Level of significance was set to 0.05.

saturated fatty acids, salts, and added sugars, have been associated with higher risk of MS [48]. In contrast, higher intake from vegetables, fruits, whole cereals, and fish and fish products were associated with a reduced risk of MS [48].

Interestingly, the associations between PS of sugar-rich products such as cakes, pies, and biscuits and sugar, honey, jams, and chocolate were associated with a higher metabolic risk score. It has been confirmed that sucrose and mainly fructose induce MS [49]. For example, in young men, serum triacylglycerol increased when receiving a diet supplemented with 200 g sucrose/day; moreover, one-third of these patients developed hyperinsulinemia [49]. In addition, consumption of sugar-sweetened beverages has the same result on MS development, which may be explained by less satiety-inducing effects and a high-glycemic-index, which raises the postprandial glucose levels [50]. The mechanism was associated to the inability of sugar to acutely stimulate insulin and leptin and to inhibit ghrelin that are known to affect the satiety center in the central nervous system [51]. Moreover, the sweetness of fructose or sucrose often makes food more palatable, which may stimulate an increase in food intake [52].

4.3. Portion size of specific food groups and HOMA-IR cutoff

Our results indicated that males with lower HOMA-IR cutoff were consuming higher mean PS from vegetables, milk, yoghurt, and milk beverages. In the same manner, females with lower HOMA-IR cutoff were consuming higher mean PS from breakfast cereals. In contrast, females with higher HOMA-IR cutoff were consuming higher mean PS from butter and animal fats. These results are in line with other studies which observed that consumption of vegetables, low-fat dairy, cruciferous vegetables, tomato, chicken, and beans and low consumption of butter, red meat, and cereals was associated with low levels of HOMA-IR in adults [53]. On the contrary, they found that consumption of processed meats, mayonnaise, and solid fats was associated with a higher level of fasting blood glucose, fasting insulin, 2-h insulin, and HOMA-IR index [53]. A previous HELENA study also found that frequently consumption of nuts, chocolates, burgers, and meat stick in females and frequently consumption of burgers and pizzas in males were directly associated with HOMA-IR index [12]. Moreover, a marked preference for sweet and low fruit consumption and IR were observed in other

Table 5 Mean PS from the main contributing food groups by HOMA-IR cut off categories, in both gender (ANCOVA).

Food groups PS (g/day)	Males (n = 230)					Females (n = 265)				
	^a HOMA-IR cut off ≤2.5		HOMA-IR cut off >2.5		p-value	HOMA-IR cut off ≤2.5		HOMA-IR cut off >2.5		p-value
	N	Mean (SD)	N	Mean (SD)		N	Mean (SD)	N	Mean (SD)	
<i>Bread and rolls</i>	146	134.1 (82.5)	42	144.2 (74.9)	0.228	166	111.3 (70.7)	67	93.1 (49.3)	0.493
<i>Breakfast cereals</i>	48	54.6 (30.5)	21	70.7 (50.3)	0.479	63	49.9 (28.6)	19	28.5 (10.8)	0.010
<i>Rice and other grains</i>	57	192.1 (135.9)	15	225.2 (117.5)	0.817	55	156.2 (95.7)	21	128.0 (75.1)	0.976
<i>Starch roots, potatoes</i>	76	159.2 (89.1)	16	158.4 (62.4)	0.993	103	142.4 (87.7)	45	123.8 (74.3)	0.244
<i>Pasta</i>	64	217.9 (109.9)	14	218.7 (105.7)	0.698	73	178.1 (73.5)	26	215.0 (113.5)	0.149
<i>Fruits</i>	98	244.1 (153.3)	26	239.8 (153.9)	0.778	121	206.2 (125.5)	43	194.1 (130.2)	0.954
<i>Vegetables</i>	127	193.4 (122.6)	37	136.4 (115.7)	0.036	149	133.6 (112.3)	63	128.2 (99.7)	0.784
<i>Milk, yoghurt, and milk beverages</i>	120	434.1 (354.6)	34	348.9 (217.7)	0.040	137	317.1 (212.9)	51	279.5 (168.4)	0.188
<i>Desserts and puddings milk based</i>	29	105.6 (74.1)	6	52.3 (24.8)	0.060	55	89.2 (68.9)	15	76.3 (45.7)	0.693
<i>Cheese</i>	119	57.4 (42.9)	31	77.9 (48.36)	0.314	123	44.3 (34.2)	52	43.6 (29.9)	0.989
<i>Meat and poultry</i>	147	237.1 (200.6)	37	260.6 (225.3)	0.265	144	161.6 (133.5)	66	178.5 (134.6)	0.331
<i>Fish and fish products</i>	30	208.5 (172.1)	5	108.6 (54.3)	0.251	30	156.3 (132.9)	13	145.9 (118.9)	0.439
<i>Eggs</i>	28	53.5 (41.5)	9	55.8 (34.1)	0.512	52	51.5 (42.6)	15	62.4 (50.1)	0.715
<i>Meat substitutes, nuts, pulses</i>	29	61.5 (57.0)	10	27.9 (10.9)	0.120	40	82.5 (76.1)	12	75.9 (56.4)	0.962
<i>Margarines and vegetable oils</i>	102	27.1 (13.4)	26	37.4 (30.1)	0.188	97	18.9 (16.4)	43	19.9 (17.1)	0.515
<i>Butter and animal fats</i>	57	28.8 (23.5)	14	22.9 (13.6)	0.496	58	18.9 (13.6)	25	27.3 (24.9)	0.018
<i>Cakes, pies, biscuits</i>	109	115.9 (84.8)	26	106.7 (78.2)	0.798	132	98.5 (78.8)	50	104.3 (101.1)	0.377
<i>Savoury snacks</i>	38	62.8 (51.3)	6	55.8 (42.9)	0.671	43	38.2 (25.2)	14	32.9 (15.5)	0.457
<i>Sugar, honey, jams, chocolate</i>	106	74.4 (62.4)	26	68.7 (55.4)	0.920	133	63.2 (50.8)	57	42.6 (36.5)	0.138
<i>Sauces and creams</i>	107	67.0 (52.8)	22	77.9 (49.1)	0.494	113	64.2 (54.1)	44	73.9 (56.3)	0.343
<i>Carbonated soft/isotonic drinks</i>	99	529.9 (325.2)	26	529.9 (416.1)	0.824	102	462.3 (309.4)	32	419.2 (262.6)	0.869
<i>Fruit and vegetables juices</i>	103	408.6 (319.7)	30	351.3 (209.3)	0.162	105	332.2 (241.5)	51	352.9 (180.1)	0.437

^a HOMA-IR cut off categorized based on the 90th percentile. SD: Standard deviation. Level of significance was set to 0.05.

study in adolescents [15]. However, to the best of our knowledge, there are no studies addressing the relationship between food PS and HOMA-IR index.

The possible explanation of these results is that vegetables, fruits, and cereals are a good source of antioxidants mainly polyphenols and fibers, in addition to the significant amount of magnesium, calcium, potassium and having a limited amount of sodium, which play an important role in IR in all age groups [54]. For instance, individuals with low levels of serum magnesium have impaired blood sugar levels [55]; it has been found that the lack of magnesium can result in disordered transfer of the cellular glucose, which influencing in insulin signaling pathways or even reducing the pancreatic secretion of insulin [56]. Furthermore, a high amount of sodium intake has been associated with IR and MS development in adults [57].

Moreover, the type and quality of fats in the diet play an important role in homeostasis and insulin sensitivity [58]. It seems that the fatty acid combination or fat type can affect independently on insulin function and lead to change the cells sensitivity to insulin [59]. However, in adolescents with obesity, a positive effect when consumption of omega-3 fatty acids on insulin sensitivity has been identified [60]. Consumption of milk and yogurt is another factor that has been examined on IR syndrome; it has been shown that the daily consumption of dairy and calcium cause a

reduction of the IR syndrome, cardiovascular disease, blood pressure, and stroke in young adults [53].

In this study, no significant relationship between food PS and continuous HOMA-IR index was found, which may be associated with the very restrictive model with various confounders. However, some relevant results when analyzing the results considering the HOMA-IR cutoff values were observed, as it was discussed previously.

Several studies have attempted to explain the association of dietary indexes on the development of IR and MS, but most of them do not focus on PS of food items. Although the effect sizes in our study were small, further studies are needed to confirm the association between food PS and the metabolic markers.

The main limitation of this study is the relatively small sample size. Further studies with larger population samples and longitudinal observation are needed. Moreover, the HELENA study was performed some years ago, and it is not useful to describe the current situation. Additionally, the cross-sectional nature of the HELENA study does not allow us to assess the behavior over a period of time and did not provide information in determining the cause-and-effect association. The self-reported questionnaires were used for collecting the food consumption data, and therefore, a social bias should be considered. Moreover, the food groups did not differentiate between type of

chocolate (black, with milk, etc) and artificially sweetened products from sugar-sweetened products. However, there are several strengths in this study that need to be mentioned. To our best of knowledge, the present study is the first to investigate the association between the PS of different food groups and both IR and metabolic risk among European adolescents, taking into account potential confounders such as age, total energy intake, BMI, PA, city, and maternal education. To increase the accuracy, highly standardized and validated procedures were used to collect the sample and assess anthropometric measurements. Despite the limitation, these results may suggest useful information to promote proper selection of healthy foods for early prevention of MS and other health concerns.

Conclusion

Larger PS from cakes, pies, and biscuits were associated with a higher metabolic risk score, and PS from fish and fish products; meat substitutes; nuts, pulses, and sugar; and honey, jams, and chocolate were associated with a higher metabolic risk score in males. Noteworthy, these results suggest that there are associations between PS of sugar-based foods and metabolic risk already in adolescence. Larger PS from vegetables, cereals, and dairy products enhance the VO₂ max and might reduce developing IR. Overall, these findings suggest that intervention studies should focus on the food PS and not only on the potential effect of food habits and energy density in order to prevent IR and metabolic risk in youth.

Author contributions

The HELENA study was designed and contributed to get the funding by Moreno. L, Gonzalez-Gross. M, Castillo. M, Molnár.D, Stehle. P, Widhalm. K, Kafatos. A, and Dallongeville.J. The supervision procedure and acquisition of data were done by Moreno. L, and Gonzalez-Gross.M. Field work contribution and data analysis were conducted by Castillo. M, Marcos. A, Gottrand. F, Huybrechts. I and the rest of authors. Molnár.D was responsible for the body composition work package. Flieh. S analyzed the data and wrote the manuscript Miguel-Berges. M, González-Gil. EM and Moreno. L critically revised the manuscript, provided essential comments, and supervised all procedures. All co-author revised the manuscript and provided their essential comments. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement

The HELENA study was approved by the ethics committees in all countries, and followed good clinical practice, ethical guidelines of the Declaration of Helsinki 1964 (revision of 2000), and the legislation about clinical research in humans in each one of the countries involved in the study. The ethical approval code from the coordinator centre was 03/2006; date of approval: February 2006, obtained from the Ethical Committee of clinical research in Aragon (CEICA).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

The data presented in this study are available for further scientific analysis on request from the coordinator of the HELENA study to the following. e-mail: Imoreno@unizar.es.

IARC disclaimer

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.numecd.2022.05.017>.

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